Protocol for conditioned media experiments with primary macrophage and SMC

1. Isolate smooth muscle cells from aorta- digest with collagenase and strip adventitia, culture on gelatin coated plates in 1 mL FBS+ 1 mL DMEM+ 4.5g glucose until SMC proliferate and attach.
2. After 1 week, change media to 10% FBS + DMEM + 4.5g. After about 2 weeks or at ~60% confluence, collect conditioned media every 2-3 days. Freeze or add directly to macrophage culture medium.
3. Isolate BMDM from animals, grow in T25 flask + 10 mL DMEM +10%FBS +10uL M-CSF. Day after isolation, split into two 5 mL T25 flasks.
4. Day 3 after isolation, add 1mL conditioned media from macrophages.
5. Day 5-6 after isolation, add another 5mL of DMEM+10%FBS+5 uL MCSF + 1mL conditioned media
6. Harvest on Day 7 or 8.